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Appraisal the Protective Effects of Cymbopogon Schoenanthus Extract against Reproductive Disorders and Carcinogenic Effects of Formalin in Experimental Male Rats

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ABSTRACT: Formaldehyde has been documented to be naturally present in many common foods. There has been a big public concern over the use of formaldehyde in the preservation of food. Also, it is commonly used as a chemical substance, usually in the life and can interact with many bio-substance in the human body. The present study target to investigate the protective effects of Cymbopogon schoenanthus (CS) extract against the reproductive and carcinogenic effects of formaldehyde on male rats. The Albino male rats were divided into equal six groups, first group: rendered as a control group; second group: received formalin (100 mg/kg bw) and third group and forth group: were received SC extract at (50 and 100 mg) respectively; fifth group and sixth group were received formalin (100mg /kg bw) + SC extract (50mg) and formalin (100mg /kg bw)+ SC extract (100 mg) respectively. At the end of the experiment the animals were scarified and blood samples were collected for measurement all tested parameters. The results showed that the oral exposure to formaldehyde at a dose of 100 mg/kg bw resulted in significant negative effects in all tested parameters, while the CS extract at tow doses (50 and 100 mg) alone or in combination with formalin restored the negative effects to normal levels compared with the untreated group. The histopathological examination was studied on testis tissues and the histopathological pictures showed the CS extract at tow mention doses had ameliorate the adverse effects that induced by formaldehyde hazards.

Keywords: Formaldehyde, Cymbopogon, Animals, Sexual hormons, Tumor.

INTRODUCTION

Formalin or a formaldehyde water solution contains (40vol% or 37wt%), Formaldehyde (FA) extremely flammable gas with a colorless properties and a very reactive compound. FA can interact with many varied macromolecules, such as proteins, nucleic acid and amino acids (low molecular weight substances) (Metz et al., 2004). FA in very low concentration induced ngative health effect in womens (Peter F et al 2019).

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Recently, it has been reported that formalin is widely used in a lot of countries as a chemical preservative for fruits and fishes products (Uddin et al., 2014). Also, FA found in photochemical smog and various fruits. On the other hand, it widely used in many industries (paper product, building, textile, resin, wood composite, insulting materials, paints, plastic, fabric, adhesive and cosmetic industries) (Trezl et al., 1998). In vivo, FA can be configuration by the metabolism of L.Methionine, histamine or methylamine, and can react with folic acid to participate in biological methylation (Trezl et al., 1998).

The International Agency for Research on Cancer proceeds many studies (IARC, 2014) had mentioned that FA acts numerous hazard on human kind "carcinogenic to humans" based on more risks factor of nasopharyngeal cancer and leukemia. Exposure to formaldehyde solution multiplied the secretion of corticotrophin liberating hormone (CRH) from the hypothalamus and involved a corticosteroid adrenal release, which decline testosterone hormone by means of influance the hypothalamus-pituitary-testis (HPT) axis (Sari DK 2014). There are traditional ways for detoxification of some food contaminants such as Amonia (Gomaa M. et al 1997)., that may representing a risk. Also the current medicine may cause side effects even greater dangerous than the disease for which they're ate up regardless of having a superficial attraction as compared to the medications used according to traditional medicine used in accordance to conventional medicinal drug, medicinal plant can exert beneficial outcomes on organs of the body with several therapeutic effects and also cause only a few side effect, if used at suitable doses (Bahmani, 2014;Delfan, 2015;Nasri, 2015; Mardani, 2015). Some plant extracts decreased the tumor marker (AFP) levels, and decreased the viral titers in hepatitis C virus patients and has a powerful therapeutic effects against hepatitis C virus and liver cancer Abdel-Wahhab, M. A. et al. (2011). The CS extract significantly suppressed the oxidative stress caused by CCl4 (Marwa M et al 2019). This study aims to evaluate the relief and protective effects of CS ethanolic extract against the hormonal imbalance, carcinogenic effects and antioxidants deficiency caused by Formalin in male rats.

MATERIAL AND METHOD

Cymbopogon schoenanthus purchased from the Egyptian Markets, Dokki, Giza. Kits of MDA, SOD, CAT were purchased from (Cayman, USA). Kits of AFP, CEA and GPx were purchased from Biodignostic Dokki, Giza (Egypt). The ELISA kits used for determination of testosterone, FSH and LH in rat serum were obtained from Glory science Co., Ltd.

Forty eight 3-months-old Dawley rats were purchased from the Animal House Colony, National Research Center, Giza, Egypt.

Two hundred grams of dried powder plant was soaked in 1000 mL ethanol (80%) and incubated at room temperature for 24 h. The slurry was filtered through a Whatman No.1 filter paper and then solvent was evaporated with a rotary evaporator at 35°C. The residue was adjusted to 100mL with distilled water and stored at 4°C.

The total polyphenolic compounds were estimated in the different C. schoenanthus extract according to Singleton and Rossi (1965) by using folin ciocalteu reagent purchased from Sigma Chemicals Co. Concentration of the total polyphenols was calculated as a gallic acid equivalent from the calibration curve of gallic acid standard solutions obtained from Sigma Chemicals Co. covering the concentration range between 0.2 and 1.0 mg / ml.

Estimation of Free radical scavenging activity for Cymbopogon schoenanthus extract DPPH radical-scavenging activity.

The percentage of the antioxidant activity was evaluated by method described

by Brand-Williams et al. (1995) using DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) for initiation of the free radicals and absorbance of the resulting solution was measured spectrophotometrically at 517 nm.

After an acclimatization period for 1 week, animals were randomly equally divided into six groups and housed in filter top polycarbonate cages. The different groups were treated for consecutive 30 days as following. First Group served control group, fed a standard diet and all others groups according to AIN 1993 (Reeves et al., 1993). The second group served as a toxins group, which treated orally with formalin (100 mg/ kg b.w), the third and fourth groups treated with SC extract at doses (50 and 100 mg/kg bw), respectively, the fifth and sixth groups were treated with formalin plus SC extract at previously mentioned doses.

At the end of the experimental period, blood samples were collected from all animals from the retro-orbital venous plexus. Serum was separated by centrifuging at 2500 rpm for 15 min and analyzed for various biochemical parameters. Then all animals were killed and the testes from each animal was excised, rinsed in ice cold 0.25M sucrose solution and 10%w/v homogenate was prepared in 0.05 M phosphate buffer (pH 7) and centrifuged at 12,000 ×g for 60 min at 4° C. The supernatant was collected is stored at −20 ˚C to be used for investigation of the expressions of SOD, CAT, GPx and MDA. Other Samples of the testes tissues from all animals were fixed in 10% neutral formalin and paraffin embedded. Sections (5µm thickness) were stained with hematoxylin and eosin (H&E) for the histological examination (Drury et al., 1980).

Quantitative estimation of testosterone was carried out in the sample of rat's serum using enzyme-linked immunosorbent assay (ELISA) according to (McCann D, Kirkish L., 1985). Quantitative estimation of FSH was carried out in the sample of rat's serum using ELISA according to (Knobil E., 1980). Quantitative estimation of LH in the sample of rat's serum was carried out using ELISA according to (Wakabayashi K., 1977).

Malondialdehyde (MDA) was analyzed by measuring the production of TBARS according to the method of Buege and Aust (1978) using a TBARS assay kit (Cayman, USA). SOD activity was determined using a Cayman SOD diagnostic kit (Cayman, USA). CAT activity was determined following the manufacturer's instructions (Aebi, 1984). While GPx was determined according to (Lawrence and Burk, 1979).

Estimation of CEA was performed by ELISA reader. The standard procedure was followed as per manufacturer's instructions for ELISA (Zamcheck et al., 1981). AFP was measured using ELISA diagnostic kits as described by manufacturer's instructions according to the principle of Gibbs et al. (1987).

For histological examination, testis tissues were dissected using paraffin wax ordinary microtechnique. Paraffin sections were cut into 6µm-thick slices and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed using an Olympus light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus E-330, Olympus Optical Co., Ltd., Japan).

Data are presented as the mean ± SD and were analyzed using SPSS statistical software (SPSS 11.0). Comparisons were performed by one-way analysis of variance or unpaired Student's t test as appropriate. $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Antioxidants substances are extremely paramount which possess the capability to improve the body from damage that incedance by free radical elevated oxidative stress.

The antioxidant activity of Cymbopogon schoenanthus extract was based on its ability to trap DPPH radical. DPPH method is the sensible way to determine the antioxidant activity of plant extracts. According to our previous publication (Sherif S., et al., 2017) we found that the amount of total plyphenols contents and DPPH radical scavenging activity of ethanolic extract of C. schoenanthus were 3.64±0.4 mg (GAE)/ml, and 76.6% respectively.

Table 1. Total polyphenols and Antioxidant activity of CS extract

Extraction	Total polyphenols (g gallic acid/ml)	Antioxidant activity (%)
Ethanolic Extract of Cymbopogon schoenanthus	3.64 ± 0.4	76.6%

In the same concern The phenolic compounds existent in plants are known for their antioxidant activity. Many studies have reported that extracts of plants that rich of polyphenols and their bioactivities contribute significantly to the antioxidant activity and act as highly effective metal chelating, lipoxygenase inhibitors and free radical scavengers which are mainly due to their redox properties (seif.et al.,2014 and 2017). antioxidants can play an paramount role in adsorbing and compensating free radicals, quenching singlet and triplet oxygen or decomposing peroxides, that reselt from esposure to environmental pollutants (seif, et al., 2017 and 2018; sherif, et al, 2017).

The effect of FA, CS extract and their combination on the FSH, LH, and testosterone hormones were measured in rat serum. The obtained results (table 3), showed that the significant negative effects on hormones leves were recorded in FA group compared to control group. While, the rats received a CS extract at tow tested doses (100 and 200 mg/kg bw) showed normal hormone levels and close to the control values. However, the coadmistration of CS extract with formalin caused significant improvement compared with FA alone. It is worthy to mention that these results confirming the safety of the CS plant extract at two mention doses asnd the ability to amorlialative effects on all tested hormones as well.

The obtained results were in agreement with the results that reported with (Zhou et al. 2006). They reported that the exposure to FA caused adverse effects on the reproductive capabilities in mice when dosed daily for two weeks. The effects found out atrophy of the seminiferous tubules, decline sperm number and sperm growing motility in the epididymis. In the same time proven that exposure to FA enhanced the secretion of corticotrophin liberating hormone (CRH) from the hypothalamus gland tissue and precipitated corticosteroid adrenal release, which reduced testosterone hormone (Sari et al., 2004).

Table 2. The effects of Formalin and CS extract on, FSH, LH, and Testosterone in male rats treated with 100 mg/kg bw formalin)

Groups	FSH (mIU/ml)	LH (mIU/ml)	Testosterone (ng/ ml)
Control	2.83 ± 0.2	1.86 ± 0.20	3.43 ± 0.20
Formalin(100mg/kg bw)	1.23 ± 0.08 *	0.93 ± 0.06*	1.07 ± 0.02 *
CS extract (100mg/kg bw)	2.46 ± 0.13	1.66 ± 0.11	3.13 ± .30
CS extract (200mg/kg bw)	2.35 ± 0.22	1.65 ± 0.15	2.93 ± 0.09
Formalin + CS (100mg/kg bw)	2.21 ± 0.37	1.64 ± 0.06	2.83 ± 080
Formalin+ CS (200 mg/kg bw)	2.25 ± 0.18	1.68 ± 0.10	2.98 ± 0.20

Data were expressed as (mean ± SD). LH: Luteinizing hormone, FSH: Follicle stimulating hormone. Analysis between groups was done using one way ANOVA test (Dennett equation). *: $P < 0.05$

Formaldehyde as a chemical component caused oxidative stress in various body tissues. oxidative stress dangerous to sperm and a tremendous element within the etiology of male infertility (Makker et al., 2009). Oxidative stress process is mischievous to sperm activity and play an paramount role in male sterility (Makker et al., 2009). Moreover, the oxidation process deleteriuos male fertility by alter the cell function activity as well as that sperm motility (Makker et al.,2009), induce damage of DNA occurring gene mutations, DNA denaturation, base pair oxidation and DNA fragmentation. The use of herbs has a long history in order to fertility regulation (Talukder et al., 2011). The role of antioxidant enzyme in most cells to avert reactive oxygen species induced injury (Sanocka et al, 1997). In the same concern, the Antioxidants in plant extracts provide the most critical defense against free radical induced male infertility (Seif, et al., 2014: Said, et al., 2005).

In the same concern, Sherif et al., 2017 suggested that C. schoenanthus extract contained (3.64 ±0.4 mg (GAE)/ml) as well as antioxidants activity reached at (76.6%). C. schoenanthus is known for its antioxidant and effective scavenging mechanism for free radicals through flavonoid and phenol (Khadri et al., 2010).

Table 3. The effects of CS extract at two different doses on tumor markers in male rats treated with 100 mg/kg bw formaldehyde).

Groups	CEA (ng/ml)	AFP (ng/ml)
Control	0.051 ± 0.02	0.18 ± 0.01
Formalin(100mg/kg bw)	0.13 ± 0.01*	0.37 ± 0.1*
CS extract (100mg/kg bw)	0.06 ± 0.01	0.21 ± 0.03
CS extract (200mg/kg bw)	0.07 ± 0.01	0.22 ± 0.01
Formalin + CS (100mg/kg bw)	0.66 ± 0.01	0.24 ± 0.01
Formalin+ CS (200 mg/kg bw)	0.06 ± 0.01	0.19 ± 0.01

Data were expressed as (mean ± SD); 1-α- fetoprotein (AFP), acarcinoembryonic antigen (CEA). Analysis between groups was done using one way ANOVA test (Dennett equation). *: $P < 0.05$

Nowadays the effect of plant-derived chemicals on endocrine and sexual organs have a great interest (Yakubu, 2008). Antioxidants may improve the sperm function and fertility, in this regard, celery extract, due to its antioxidant contents has been pointed for inhibiting the liposomal peroxidation (Popovic, 2006). The effect of traditional medical herb on male reproductive functions is related with antioxidant potency (Boudou et al., 2013; Seif et al.,2014). Also, (Elumalai, et al., 2009) reported that the phytomedicines improve male fertility due to antioxidants. Furthermore the antioxidants enhance various male reproductive functions such as steroidogenesis, spermatogenesis. For example Lycium barbarum plant restored the testicular mouse cells induced by H_2O_2 to the normal structure . Antioxidants substances improve the most critical defense against to oxidation process occurred and reduced male infertility (Said, et al., 2005; 2014).

The results summarized in the table 3 showed the effects of FA and CS extract at two different doses (100 and 200 mg/kg bw) alone or in combination with FA on serum tumor markers in rats. The results cleared that the effect of the CS extract of two different doses on the tumor markers CEA and AFP were in normal range and had no significant change compared with control results. At the contrary the rats received FA alone orally at (100mg/kg bw) had a significant elevation in tested tumor markers compared with them in control group. It is worthy to mention that the CEA and AFP tumor markers for rats received formalin plus CS extract at two different doses (100 and

200 mg/kg bw) were restored and closed with the control group. The (IARC, 2006) reported that there is sufficient evidence for the carcinogenicity of formaldehyde. The phytochemicals have the ability to inhibit cancer cytogenesis by suppressing the tumor initiation or promotion (Manosroi et al., 2006). The anticancer of cymbopogan grass has been well enumerated in the literature (Thangam et al., 2014).

Table 4. The effects of CS extract at two different doses on antioxidants enzymes in male rats treated with 100 mg/kg bw Formaline)

Groups	MDA (nmol/g tissue)	CAT (U/mg tissue)	SOD U/mg tissue)	GPx (mg/g tissue)
Control	80 ± 3.5	16.5 ± 0.8	49.34 ± 6.1	30 ± 1.2
Formalin (100mg/kg bw)	170 ± 7.2*	15.2 ± 0.9	30.3 ± 1.8*	21.4 ± 0.8*
CS extract (100mg/kg bw)	87 ± 6	15.8 ± 0.1	47.6 ± 3.4	28.3 ± 2.6
CS extract (200mg/kg bw)	84 ± 9.2	16 ± 0.15	46 ± 2.8	25.3 ± 1.7
Formalin + CS (100mg/kg bw)	97 ± 5	16.3 ± 1.3	37.3 ± 0.8	27 ± 0.5
Formalin+ CS (200 mg/kg bw)	89 ± 6.6	15.7 ± 0.4	44.7 ± 2	28.6 1.4

Data were expressed as (mean ± SD). SOD: Superoxide dismutase, GPx: Glutathione Peroxidase, CAT: Catalase, MDA: Malondialhyde; Analysis between groups was done using one way ANOVA test (Dennett equation). *: $P < 0.05$.

The antioxidants enzymes and lipid peroxidation were estimated in testes tissue homogenate. The data represented in table 4 showed that the SOD, GPx, CAT and MDA activities of control rats were in normal levels while, the rats treated with FA (100mg/kg bw) manifested significant decrease ($p>0.05$) in SOD, GPx, CAT activity and significant increase ($p>0.05$) in MDA level. While, the rats treated with SC extract at tow mentions doses widthed evenness with untreated rats in antioxidant enzymes and MDA as well. Otherwise, the rats remedied with combination of CS extract plus FA widthed significant improvement ($p>0.05$) in SOD, GSH, CAT and MDA as well. The obtained results were agreed with the results reported by (Rasyidah et al., 2014; Teng et al., 2001), they found that the exposure to FA caused significant elevation of MDA level in liver and testes tissues and significant decrese in GPx, SOD and CAT.

The finding in current study has cleared that the orall dose of FA at (100 mg/kgbw) for consecutive four weeks lead to elevated the standared of MDA in testis of rats, thses results consistent with results published with by Zhou et al. (2006) furthermore,during metabolized lipid produce lipid peroxidation process, MDA augmentation significantly in the testis of rats vulnerable to formaldehyde vapour (10mg/m3 for 2 weeks) contrast to the control group.

Also, Ozen et al. (2008) detected the excess of concentration of MDA which described impairment effect of FA on testis. The gained results in current study are acceptable with the findings of the previous studies. Recently, many studies proposed that FA can pass to the blood barrier thus release oxidative stress by induce free radicales react within the testis tissue (Vosoughi, et al., 2012). These fundamental reactive oxygen species (ROS) may be ultimately release histopathology damage in seminiferous tubules Ozen et al. (2008), sperm motility and sperm count (Kose et al., 2012) meanwhile eventually will elevate sterility in male. In spite of MDA concentricity grade in the groups exposure to FA donated oral dose of CS extract is significantly reduced as matched to the group of FA exposure only, it might be suggested that CS extract has contain antioxidant compounds effected against testicular oxidative stress by FA.

As the free radicals are permanently produced and reacted with testis tissue to make damage, in order to these tissue cells must be protected by internal antioxidants

like SOD, CAT and others. But, in the existence of the tremendous level of free radicals, supplemental external antioxidants are required to countervail the tissue cell damage effect. These exogenous antioxidants can be formed in herbal plants, such as polyphenols, vitamins and others. In the current study, the protective role of CS extract as a highly antioxidant agent was studied. All groups of experimental rat excluding Formaldyde group resulted in significant excess of SOD activity in rat's the testis SOD activities were significant decrease in formaline group compared with others tested croups. This perhaps elucidated by the physiological of testicular cell mechanisms itself. Testicular cell mechanisms, notably Spermatogenesis required highly oxygen consumption of mitochondria, it may lead to exceedingly low oxygen tension resulted to low vascularization of the testicle tissue.

In theory, the production or development of mature spermatozoa and Leydig cell steroidogenesis would be more oversensitive to an oxidation process due to the profusion of highly unsaturated fatty acids as a component of cell structure also xanthine- and NADPH-oxidases enzymes and mitochondria generating ROS. On the contrary, in order to the testicles protects itself from the risk, it has boosted a advanced antioxidant enzymatic systems encompass both superoxide dismutase, glutathione peroxidase and glutathione-S-transferase. Either, non-enzymatic antioxidant agents play important role such as vitamin C and E, zinc, melantonin and cytochrome C components (Aitken and Roman, 2008). So, it is noticed that high antioxdant activity of SOD in these empirical groups because the testicular tissue itself contain not only cytosolic (Cu/Zn) and mitochondrial (Fe/Mn) forms of SOD but also merit special form of SOD exist extracellular which is produced by both sertoli and germ cells (Aitken and Roman, 2008). In addition, we exposed that there is specific contribution of SOD from the CS extract lead to significant induce of SOD activity in groups ingested orally CS extract only as matched with a control group. This is produce evidence that the ethanolic extract of CS has an antioxidant activity effect which confirmed by a study done by (Sherif et., 2017). No doubt, In the existence of SOD, superoxide anion (O_2-) is fast turned to hydrogen peroxide (H_2O_2) in order to protect the former from involving in the formation of highly pernicious hydroxyl radicals. The elimination of H_2O_2 is either affected by catalase (CAT) or glutathione peroxidase (GPx), with the latter predominating in the case of the testicles (Aitken and Roman, 2008). in spite of, catalase is of finite importance role in the testes tissue cells, it has remained a vital antioxidant enzyme to remove toxic hydrogen peroxide. As noticed in the current study that no significant difference in catalase activity pay attention in all empirical groups as compared to control group. Already, it is might be regard to turning hydrogen peroxide has been taken by enzyme GPx which are a considerable in testes cells and also catalyzing hydrogen peroxide to oxygen and water (Aitken and Roman, 2008). However, the groups of formalin exposure treated with CS extract at tow mention doses have higher catalase enzyme activity as compared to the group of formalin only, proved that CS extract has the ability to elevate CAT activity in formalin-stressed testicles. The hormons and biochemical parameters were confirmed by histopathological study and the results (figure1) indicated that the testicular section of control group showing normal seminiferous tubules with complete spermatogenesis (1); while the second group, which treated with formalin alone showing massive edematous fluid in-between seminefrous tubules (arrow) (2); also section 3 for the third group, which treated also with FA, showing dilated and congested blood vessels (arrows). sections 4

and 5 for the rats received the CS extract at two tested doses showing normal seminiferous tubules with complete spermatogenesis while sections 6 and 7 for the rats received formalin plus-SC at two tested doses showing restored and improvements in histopathological archticture compared with sections 2 and 3. our finding similar to results was found by (Vosoughi et al., 2012) they found that exposure to formaldehyde vapor can destroy testicular structure and decrease percentages of sperm count and progressive motility in the same concern (Adegbegi and Oso, 2015) reported that ethanolic and aqueous extracts (200mg/kg body weight) of Cymbopogon citratus was safe and has no adverse effects on the examined organs.

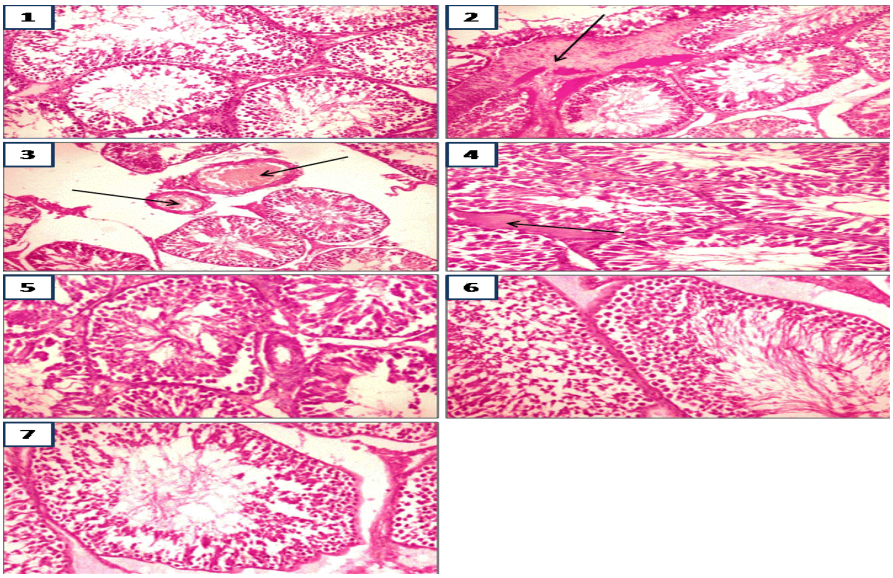

Fig. 1. Micrograph of a rat testis tissue in different groups treated with FA and CS extract and in combination

CONCLUSION

According to the findings, it can be concluded that the negative effects of formalin are noted on antioxidant enzymes, testis hormones and tested tumor markers in rats. It is worthy to mention that, the alterations in these tested parameters were ameliorated by coadministration with CS extract apparently due to its antioxidant properties activity.

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CONFLICT OF INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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